Changes in Tissue Biochemical Composition and Energy Reserves Associated With Sexual Maturation in the Ommastrephid Squids *Illex coindetii* and *Todaropsis eblanae*

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Abstract. The aim of this study was to investigate the biochemical changes that occur during sexual maturation of the squids *Illex coindetii* and *Todaropsis eblanae*. In both species, amino acids and protein content increased in the gonad throughout maturation, but the allocation of these nitrogen compounds from the digestive gland and muscle was not evident. A significant ($P < 0.05$) increase in the content of lipids and fatty acids was observed in the gonad and digestive gland. It seems that both species take energy for egg production directly from food, rather than from stored products. Analyses for cholesterol revealed a significant ($P < 0.05$) increase in the gonad, and the lipid content differences between species are potentially related to different feeding ecologies. The glycogen reserves in the gonad increased significantly ($P < 0.05$), suggesting that glycogen has an important role in the maturation process. It was evident that sexual maturation had a significant effect upon the gonad energy content, but because the energy variation in the digestive gland and muscle was nonsignificant ($P > 0.05$), there was no evidence that storage reserves are transferred from tissue to tissue.

Introduction

Illex coindetii (Vérany, 1839) and *Todaropsis eblanae* (Ball, 1841) are neritic ommastrephid squids that are caught near the bottom on the continental shelf and slope of the Atlantic Ocean, where they are voracious opportunistic

predators and important members of food webs (Clarke, 1966). Though these cephalopod species are commercially important by-catches of commercial demersal trawls in the east Atlantic (Lordan *et al.*, 1998; Robin *et al.*, 2002), several aspects of the biology and ecology remain poorly studied, including the reproductive biology (Laptikhovsky and Nigmatullin, 1999; Hernández-García, 2002). However, according to Rocha *et al.* (2001), both these squids seem to have an intermittent terminal spawning; *i.e.*, the spawning is monocyclic and eggs are laid in separate batches during the relatively long spawning period. According to Calow (1973), terminal spawning species (semelparous strategy) invest more in their single spawning event than do multiple spawning species. In fact, the complete dedication of energy to reproduction results in a terminal spawning event; in contrast, a partial cost both before and during reproduction allows individuals to spawn repeatedly throughout their adult life. Moreover, the reproductive strategy exhibited by a species is inextricably linked to its source of reproductive energy (feeding or storage) (Jackson *et al.*, 2004).

Sexual maturation and reproduction influence the status of a number of physiological processes and consequently the animal's ecology and behavior. Clarification of the energy storage and consumption patterns of these squids will shed light on the interaction between organism and environment. Cephalopods have a protein-based metabolism (Lee, 1994), and the direct use of protein as an energy reserve may account for the lack of major reserves of glycogen and lipid in cephalopod tissues (O'Dor *et al.*, 1984; Storey and Storey, 1983). The lipids in the digestive gland have also been hypothesized to be possible substrates

and a site for energy storage in cephalopods (O'Dor and Webber, 1986; O'Dor and Wells, 1978; Moltschaniwskyj and Semmens, 2000).

Here, we focus on the changes in biochemical composition that take place in the gonad, digestive gland, and muscle of *Illex coindetii* and *Todaropsis eblanae* during sexual maturation. Specifically, we examine the content of lipid, glycogen, cholesterol, and energy in these tissues, as well as the profiles of total amino acid and fatty acid.

Materials and Methods

Samples

Specimens of *Illex coindetii* and *Todaropsis eblanae* were collected off the Portuguese west coast (Peniche) by commercial trawlers and off the south coast (Algarve) on several cruises aboard the R/V *Noruega* and R/V *Capricórnio* of Instituto de Investigação das Pescas e do Mar (IPI-MAR) in February, March, April, and June of 2002. For each animal, the following parameters were recorded: mantle length, total weight, gonad weight, digestive gland (also called "liver" in some biochemical literature) weight, and depth range of catch (Table 1). For both species, maturity stages were determined following Lipinski (1979), and the specimens were classified as immature (stages 1 and 2) or mature (stage 5). Gonadosomatic index (GSI—gonad wet weight/body wet weight, $\times 100$) and digestive gland index (DgI—digestive gland weight/ body wet weight, $\times 100$) were also determined. Each of the tissue types collected (gonad, digestive gland, and muscle) were pooled, after freeze-drying in a Savant VP100®. The biochemical analyses were performed in triplicate in these tissues.

Protein and amino acid analyses

Protein concentration was determined (with 100 mg of wet tissue) on the washed TCA precipitate solubilized in $1M$ sodium hydroxide (NaOH) for 24 h as described by Lowry *et al.* (1951), using the Bio-Rad protein assay (BIO-RAD). Bovine gamma globulin (BIO-RAD) was used as a standard.

To determinate the total amino acid profile, proteins were hydrolyzed with 6 N hydrochloric acid (containing 0.1% phenol) in a MLS-1200 Mega Microwave System (Milestone), at 800 W, 160 °C for 10 min. The hydrolysis was performed under inert and anaerobic conditions to prevent oxidative degradation of amino acids. The hydrolysates were filtered and dissolved in sodium citrate buffer, pH 2.2. Amino acids were separated by ion exchange liquid chromatography in an automatic analyzer Biochrom 20 (Amersham Biosciences), equipped with a column filled with a polysulfonated resin (250 $\times$ 4.6 mm), using three sodium citrate buffers—pH 3.20, 4.25, and 6.45 (Amersham Biosciences)—and three temperatures (50 °C, 58 °C, and 95 °C). The detection of amino acids was done at 440 nm and 570 nm after reaction with ninhydrin (Amersham Biosciences). Amino acids were identified by comparison of their retention time with those of specific standards (Sigma) and quantified with the software EZChrom Chromatography Data System, vers. 6.7 (Scientific Software Inc.) using norleucine (Sigma) as internal standard.

Total lipids and fatty acid analyses

Total lipids were extracted by the method of Bligh and Dyer (1959). The fatty acid profile was determined using the experimental procedure of Lepage and Roy (1986) as modified by Cohen *et al.* (1988). The fatty acid methyl esters were analyzed in a Varian 3400 gas chromatograph equipped with an auto-sampler and fitted with a flame ionization detector. The separation was carried out with helium as carrier gas in a fused silica capillary column Chrompack CPSil/88 (50 m $\times$ 0.32 mm id), programmed from 180 °C to 200 °C at 4 °C min^{-1}, held for 10 min at 200 °C, and heated to 210 °C for 14.5 min, with a detector at 250 °C. A split injector (100:1) at 250 °C was used. Fatty acid

Table I

Summary of biological data for Illex coindetti *and* Todaropsis eblanae

Species	Sex	Maturation stage	n	ML (mm)	TW (g)	GW (g)	DgW (g)	GSI	HSI	Collection depth range (m)
Illex coindetii	Males	Immature	17	104.12 ± 9.08	31.30 ± 8.19	0.64 ± 0.40	1.42 ± 0.49	2.04 ± 1.25	4.56 ± 0.99	30–520
		Mature	14	132.00 ± 6.78	89.50 ± 23.60	4.25 ± 0.72	4.18 ± 1.16	4.86 ± 0.59	4.75 ± 0.91	30–520
	Females	Immature	18	117.76 ± 18.43	40.51 ± 18.79	0.31 ± 0.12	2.04 ± 1.30	2.41 ± 0.33	4.85 ± 1.20	50–500
		Mature	12	184.50 ± 26.73	199.71 ± 60.53	30.56 ± 3.43	12.70 ± 1.98	11.42 ± 0.60	4.72 ± 0.17	50–500
Todaropsis eblanae	Males	Immature	14	98.04 ± 13.87	82.20 ± 39.73	3.70 ± 2.60	4.81 ± 2.82	3.94 ± 1.24	5.90 ± 2.88	80–520
		Mature	11	127.30 ± 16.44	124.57 ± 17.06	6.81 ± 1.43	5.61 ± 2.89	5.53 ± 1.20	4.44 ± 2.16	80–520
	Females	Immature	10	96.12 ± 18.49	73.39 ± 39.89	0.33 ± 0.41	3.81 ± 1.93	0.34 ± 0.22	5.91 ± 2.60	80–520
		Mature	8	166.63 ± 18.21	290.32 ± 89.30	18.67 ± 8.89	20.45 ± 6.81	5.74 ± 2.13	6.43 ± 0.72	80–520

Abbreviations: *n*, number of individuals; ML, mantle length; TW, total weight; GW, gonad weight; DgW, digestive gland weight; GSI, gonadosomatic index; HIS, hepatosomatic index.

methyl esters were identified by comparing their retention times with those of chromatographic Sigma standards. Peak areas were determined using the Varian software.

Cholesterol analyses

The quantification of cholesterol content was based on the experimental procedure of Naemmi *et al.* (1995) as modified by Oehlenschläger (2000). The cholesterol was analyzed in a Hewlett Packard 5890 gas chromatograph. The separation was carried out with helium as carrier gas in an HP5 column (30 m $\times$ 0.5 mm id). The temperatures of the oven, injector, and detector were 280 °C, 285 °C, and 300 °C, respectively. Cholesterol was identified and quantified by comparison with standards (Sigma) from which a standard curve was prepared.

Glycogen analysis and bioenergetic calculation

Glycogen concentrations were determined according to the method described by Viles and Silverman (1949). Tissue samples were boiled with 1 ml of 33% potassium hydroxide for 15 min. After cooling, 50 μl of a saturate sodium sulfate solution and 2 ml of 96% ethanol were added. Samples were placed in an ice bath for precipitation (~30 min). Following centrifugation, the precipitate was dissolved in 0.5 ml of distilled water, again precipitated with 1 ml of ethanol, and redissolved in 0.4 ml of distilled water. Glycogen was then measured by the anthrone-reagent method (72 ml of concentrated sulfuric acid was added to 28 ml of distilled water, 0.05 g of anthrone, and 0.05 g of thiourea; the mixture was heated at 90 °C for 20 min) and the absorbance read at 620 nm. A calibration curve was prepared with a glycogen (Sigma) standard.

The energy content was estimated according to Winberg (1971), using factors of 12.6, 12.1, and 39.3 J mg^{-1} for protein, carbohydrate, and lipid, respectively. It is worth noting that the carbohydrate fraction was underestimated since it included only the glycogen content.

Statistical analysis

Data were analyzed using an ANOVA. Previously, normality and homogeneity of variances were verified by Kolmogorov-Smirnov and Bartlett tests, respectively. Having demonstrated a significant difference somewhere among the groups with ANOVA, we applied the Tukey test to find out where those differences were (Zar, 1996).

Results

Biological data

The mean mantle length, total weight, gonadosomatic index, and digestive gland index of *Illex coindetii* and *Todaropsis eblanae* are shown in Table 1. The gonadoso-

matic index increased through the oogenesis and spermatogenesis of both the species, but the digestive gland index showed an unclear variation trend.

Protein and amino acid content

The protein content in the gonad, digestive gland, and muscle of *Illex coindetii* and *Todaropsis eblanae* is presented in Figure 1. Both species showed an increase of these nitrogen compounds in the gonad throughout sexual maturation, the highest values being obtained during oogenesis
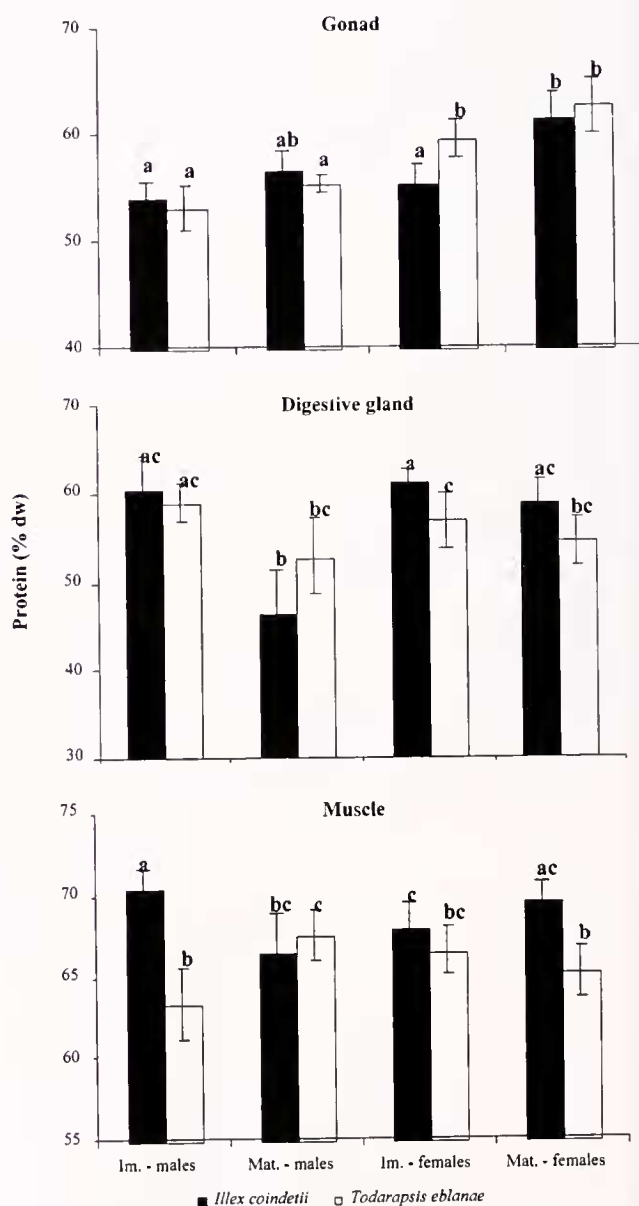
Figure 1. Protein content (% dry weight) in the gonad, digestive gland, and muscle of *Illex coindetii* and *Todaropsis eblanae* males and females at different stages of gonad development. Means ± SD with different letters represent significant differences ($P < 0.05$). Im, immature; Mat, mature.

(from 55.26% to 61.54% dry weight in *I. coindetii* and from 59.64% to 62.69% dryweight in *T. eblanae*) ($F_{7,16} = 11.23$, Tukey test $P < 0.05$). The amino acid analyses corroborated these findings. The total (protein bound + free) amino acid composition in the gonad is shown in Appendix Table 1. The major essential amino acids were arginine, leucine, and lysine. The major nonessential amino acids were glutamic acid, aspartic acid, and alanine. In the digestive gland, the protein content decreased significantly only during spermatogenesis of *I. coindetii* ($F_{7,16} = 24.39$, Tukey test $P < 0.05$). The major essential amino acids were also arginine, leucine, and lysine; the major nonessential amino acids were glutamic acid, aspartic acid, and alanine (Appendix Table 2). In the muscle, both protein and amino acid analyses revealed an unclear variation throughout the maturation process of both species. The major essential and nonessential amino acids in the muscle were the same found in gonad and digestive gland (Appendix Table 3).

Lipid and fatty acid content

The lipid contents in the gonad, digestive gland, and muscle of *Illex coindetii* and *Todaropsis eblanae* are shown in Figure 2. A significant increasing trend was observed in the gonad during the maturation of both species (mainly during oogenesis), and between species, higher values were always attained in males and females of *T. eblanae* ($F_{7,16} = 32.22$, Tukey test $P < 0.05$). Similar results were obtained with the fatty acid analyses (Appendix Table 4). Most of the saturated fatty acid content was represented by 16:0 and 18:0, monounsaturated fatty acid content by 18:1 and 20:1, and polyunsaturated fatty acid content as arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3). Between the different tissues analyzed, the highest lipid and fatty acid levels were obtained in the digestive gland and the lowest in the muscle. In the digestive gland, a significant increase in the lipid (Fig. 2) and fatty acid (Appendix Table 5) content was observed during maturation, primarily during oogenesis (lipids and fatty acids: $F_{7,16} = 21.79$ and 29.67, Tukey test $P < 0.05$). In the muscle, the lipid content showed an unclear trend during maturation (Fig. 2) and, as in the other tissues, the major fatty acids in the muscle were 16:0, 18:0, 18:1, 20:1, 20:4n-6, 20:5n-3, and 22:6n-3 (Appendix Table 6).
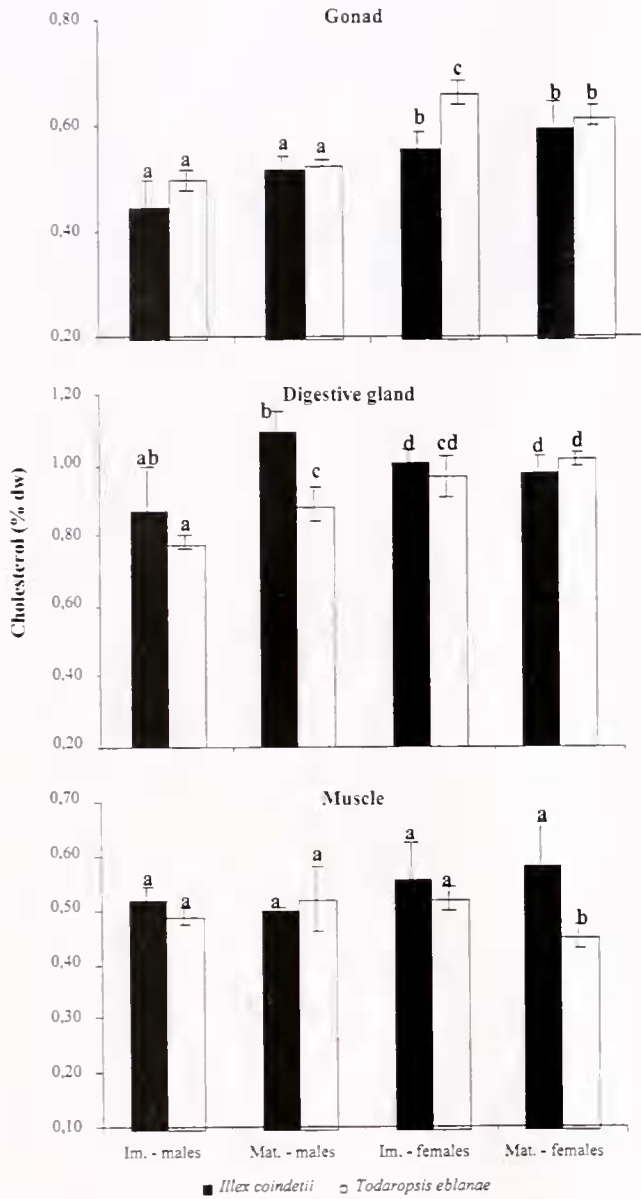
Cholesterol, glycogen, and energy content

The cholesterol content in the gonad, digestive gland, and muscle of *Illex coindetii* and *Todaropsis eblanae* is shown in Figure 3. In the gonad, the differences during sexual maturation were not significant, with the highest values being attained by females. Between species, *T. eblanae* always had the highest values in both sexes ($F_{7,16} = 42.36$,
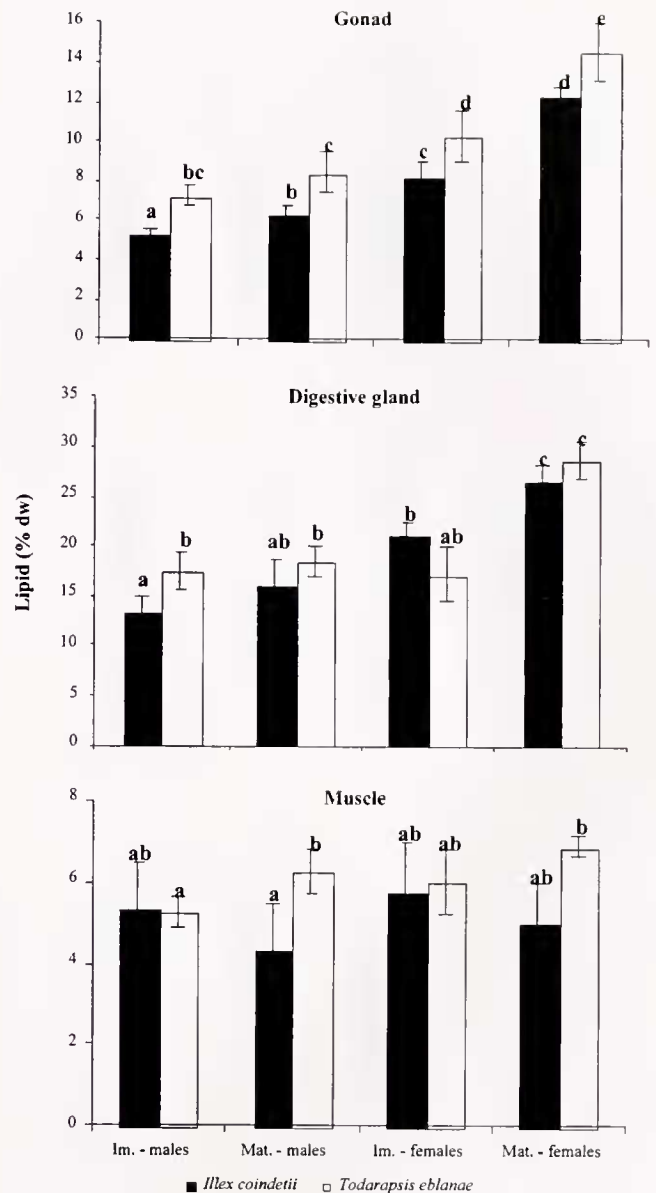
Figure 2. Lipid content (% dry weight) in the gonad, digestive gland, and muscle of *Illex coindetii* and *Todaropsis eblanae* males and females at different stages of gonad development. Means ± SD with different letters represent significant differences ($P < 0.05$). Im, immature; Mat, mature.

Tukey test $P < 0.05$). In the digestive gland, although there were significant differences between immature and mature males of both species, the differences throughout oogenesis were not significant. The muscle presented lower cholesterol values in relation to other tissues and revealed no clear pattern of variation throughout spermatogenesis and oogenesis. The glycogen content in the different tissues of *I. coindetii* and *T. eblanae* is shown in Figure 4. In the gonad, the glycogen content increased during maturation of both species. Trends of variation in the digestive gland and muscle were unclear. The energy content in the gonad,
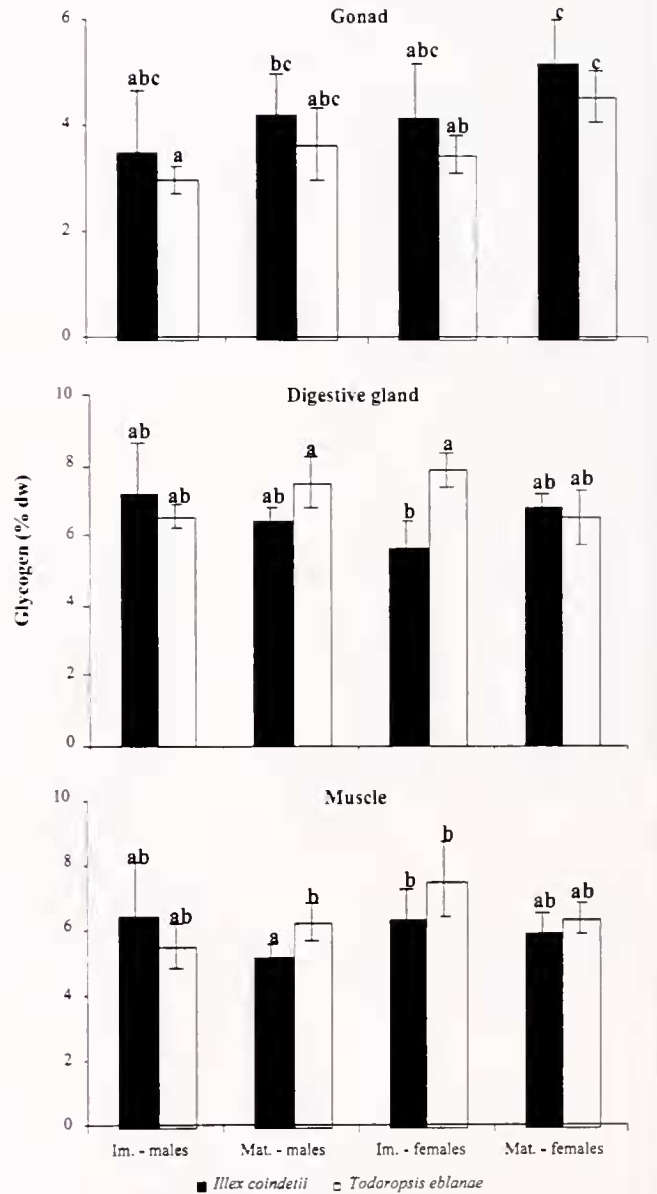
Figure 3. Cholesterol content (% dry weight) in the gonad. digestive gland. and muscle of *Illex coindetii* and *Todaropsis eblanae* males and females at different stages of gonad development. Means ± SD with different letters represent significant differences ($P < 0.05$). Im, immature; Mat. mature.

digestive gland. and muscle of *I. coindetii* and *T. eblanae* is shown in Figure 5. There was a significant increase in the gonad energy content during sexual maturation of both species ($F_{7,16} = 32.36$. Tukey test $P < 0.05$). In the digestive gland. the energy content differences did not seem to be related to sexual maturation ($F_{7,16} = 26.18$. Tukey test $P < 0.05$). In the muscle there was no significant variation between species. sexes. and maturation stages ($F_{7,16} = 2.52$. $P > 0.05$).

Discussion

In the present study, we observed a clear increase in the gonadosomatic index, but there was not a concomitant decrease in the digital gland index, which suggests that the digestive gland resources are not depleted and if resources are mobilized from this organ, then these resources seem to be compensated by those gained from feeding. The digestive gland may continue to accumulate substantial reserves of energy throughout sexual maturation, with no evidence

Figure 4. Glycogen content (% dry weight) in the gonad. digestive gland. and muscle of *Illex coindetii* and *Todaropsis eblanae* males and females at different stages of gonad development. Means ± SD with different letters represent significant differences ($P < 0.05$). Im, immature; Mat. mature.
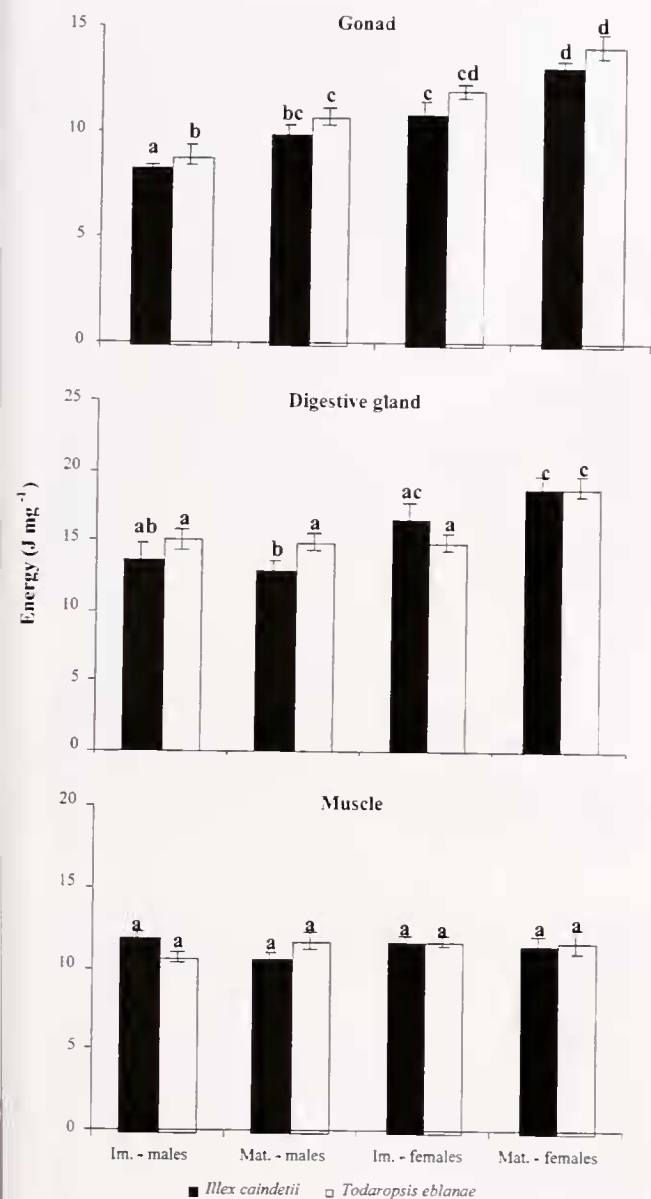
Figure 5. Energy content (J mg^{-1}) in the gonad, digestive gland, and muscle of *Illex coindetii* and *Todaropsis eblanae* males and females at different stages of gonad development. Means ± SD with different letters represent significant differences ($P < 0.05$). Im, immature; Mat, mature.

for the utilization of either digestive gland or mantle tissues to supply energy for gonads. In fact, other studies have shown that ommastrephids and other species maintain their condition, continue to growth, and fuel their reproduction from feeding rather than energy stores (Harman *et al.*, 1989; Rodhouse and Hatfield, 1990; Clarke *et al.*, 1994; Gabr *et al.*, 1999; McGrath and Jackson, 2002).

Being fast-swimming organisms, squids may not be able to utilize muscle tissue to fuel the production of gametes. However, according to Moltschaniwskyj and Semmens (2000), some species may utilize mantle tissue immediately

before spawning. More recently, in a study of the deepwater squid *Moroteuthis ingens*, Jackson *et al.* (2004) found that water tended to increase in the mantle, while protein, lipid, and carbohydrate all decreased with maturity, suggesting a transfer of energy from the muscle tissue during reproductive maturation. Additionally, it is noteworthy that in some gonatid squids, the proportions of the body and the integrity of the mantle do not change in males during maturation, but do change in females. As an adaptation for a deepwater bathypelagic "brooding" of the negatively buoyant egg, some of the female's muscle tissue degenerates (gelatinizes) (Arkhipkin and Bjorke, 1999). According to Seibel *et al.* (2000), the high lipid content of the digestive gland in females provides fuel to support the extended "brooding" period of these gonatids.

Because we used only specimens in stages 1, 2 (immature) and 5 (mature) in the present study, we could not apply a size-independent method such as geometric mean regression (see McGrath and Jackson, 2002; Jackson *et al.*, 2004), to determine how somatic condition changed with maturity. Studies on captive ommastrephids will also be needed to resolve the important life-history question of whether somatic reserves are converted into gametes.

Both squid species showed an increase of amino acids and protein content in the gonad throughout sexual maturation (mainly in oogenesis), but the allocation of these nitrogen compounds from the muscle (protein catabolism) was not evident. In addition, there was little evidence that accumulated lipid storage products were being used for egg production.

A significant increasing trend in the lipid and fatty acid contents in the gonad and digestive gland was observed, mainly in oogenesis, which suggests that, for successful reproduction, females require higher energetic resources directed towards gonad development. Moreover, it is worth noting that sex-related biochemical differences can be due to distinct feeding ecologies. Female ommastrephids are typically larger than males and are perhaps able to feed on different prey; that is, they may take more fast-swimming, oily fish, while males may take slower crustaceans with lower lipid content.

Both *Illex coindetii* and *Todaropsis eblanae* seem to use energy for egg production directly from food, rather than from stored products. This direct acquisition has also been proposed by other authors for temperate (Guerra and Castro, 1994; Collins *et al.*, 1995) and tropical (Moltschaniwskyj and Semmens, 2000) squids. Some lipid classes, namely phospholipids, have an important role in the composition of the gametes (Pollero and Iribarne, 1988), because they are probably involved in the synthesis of the vitellus, which is a yolk phospholipoprotein (Fujii, 1960). Squid gonad fatty acids were dominated by polyunsaturated fatty acids and highly unsaturated fatty acids from the n-3 series (Sargent *et al.*, 1999). Eicosapentaenoic acid and arachidonic acid are

important as structural components of cell membranes and as precursors of prostaglandins (Lilly and Bottino, 1981). Docosahexaenoic acid may be important for the correct development and survival of fast-growing phospholipid-rich cephalopod paralarvae (Navarro and Villanueva, 2000, 2003). Lipid functions in the squid life cycle must include supply of essential fatty acids and membrane components like phospholipids and cholesterol to cells.

Levels of lipid, fatty acid, and cholesterol were significantly higher in digestive gland than in gonad and muscle. The digestive gland is a site of digestive absorption and intracellular digestion (Boucher-Rodoni *et al.*, 1987; Semmens, 2002), and the differences between the two squid species may be related to different feeding ecologies. In fact, comparison between stomach content and lipid analysis confirms that lipid from digestive gland is very likely to derive from the diet with little or no modification prior to deposition (Hayashi *et al.*, 1990, Phillips *et al.*, 2001). Moreover, recent findings for the southern pygmy squid *Idiosepius notoides* have shown the presence, in the digestive gland and cecum, of intracellular lipidic droplets that derive from that squid's field diet (Eyster and van Camp, 2003).

Voogt (1973) reported that cephalopods are able to synthesize sterols, although molluscs in general apparently can only carry out this biosynthesis slowly (Goad, 1978). According to Kanazawa (2001), cephalopods seem to incorporate acetate and mevalonate into sterols poorly and then require additional dietary sources of sterol for growth and survival. Squid are active carnivores, and if their component sterols are of a dietary origin, considerable variation in the cholesterol content of the digestive gland of *Illex coindetii* and *Todaropsis eblanae* might be expected on the basis of the sterol composition of their prey. Apsimon and Burnell (1980) and Ballantine *et al.* (1981) found an exogenous rather than endogenous origin for the component sterols of *Spirula spirula* and *Illex illecebrosus*.

The cholesterol content in the gonad of the two squid species exhibited variations that seem to be correlated with the maturation process. Cholesterol is known to be an important precursor of steroid hormones, molting hormones, bile salts, and vitamin D (Kanazawa, 2001). It has been also demonstrated, in other invertebrates, that exogenous cholesterol is converted to sex hormones such as pregesterone, 17 α-hydroxyprogesterone, androstenedione, and testosterone, and to molting hormones such as 20-hydroxyecdydone (Kanazawa, 2000). Our present knowledge about carbohydrate metabolism is mainly confined to studies involving muscle work (see Storey and Storey, 1983). Cephalopods can utilize carbohydrate, lipid, and protein as metabolic substrates: lipid and protein fuel routine aerobic metabolism, and carbohydrate provides the substrate for burst (anaerobic) muscle activity (Wells and Clarke, 1996). During maturation, the glycogen reserves in the gonad increased

significantly. As it is in other marine organisms, glycogen may be important for maturation and embryogenesis. Carbohydrates are precursors of metabolic intermediates in the production of energy and nonessential amino acids and are a component of decapod ovarian pigments (Harrison, 1990).

Summing up the findings of this study, it is evident that sexual maturation had a significant effect upon the energy content of the gonads of *Illex coindetii* and *Todaropsis eblanae*, because it is associated with an increase in the products of biosynthesis that will support the lecithotrophic strategy of paralarvae. The energy variation in the digestive gland and muscle was nonsignificant, and because the digestive gland, gonad, and muscle constituents varied independently of one another, revealed no evidence that storage reserves were transferred from organ to organ. The biochemical composition of digestive gland and muscle may be influenced not by sexual maturation but instead by other biotic factors, such as feeding activity, food availability, and spawning.

Acknowledgments

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Appendix

Supplementary data on composition of *Illex coindetii* and *Todaropsis eblanae* males and females at different stages of gonad development

Appendix Table 1. *Total (protein bound + free) amino acid composition (% dry weight) in the gonad*

Amino acids (% dw)	Illex coindetii				Todaropsis eblanae			
	Males		Females		Males		Females	
	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature
Essential (EAA)								
Threonine	2.17 ± 0.12^a	2.64 ± 0.09^b	2.82 ± 0.01^c	2.91 ± 0.04^d	2.50 ± 0.11^{bc}	2.46 ± 0.09^c	2.71 ± 0.04^b	2.92 ± 0.06^d
Methionine	1.43 ± 0.00^a	1.48 ± 0.02^b	1.86 ± 0.04^c	1.69 ± 0.14^{cd}	1.46 ± 0.07^{ab}	1.52 ± 0.04^b	1.59 ± 0.03^d	1.71 ± 0.04^{cd}
Isoleucine	2.18 ± 0.10^a	2.47 ± 0.06^b	3.44 ± 0.03^c	3.16 ± 0.13^d	2.35 ± 0.06^b	2.40 ± 0.04^b	2.55 ± 0.05^e	2.75 ± 0.11^f
Leucine	3.65 ± 0.18^a	3.71 ± 0.10^a	5.26 ± 0.04^b	4.94 ± 0.14^c	3.61 ± 0.11^a	3.99 ± 0.07^{de}	3.93 ± 0.08^d	4.23 ± 0.17^e
Valine	2.17 ± 0.08^a	2.24 ± 0.06^a	2.80 ± 0.02^b	2.91 ± 0.01^c	2.23 ± 0.07^a	2.39 ± 0.08^d	2.43 ± 0.03^d	2.61 ± 0.08^e
Phenylalanine	2.91 ± 0.04^a	2.69 ± 0.06^b	3.59 ± 0.00^c	3.38 ± 0.17^d	2.63 ± 0.08^b	3.02 ± 0.09^c	2.86 ± 0.03^a	3.08 ± 0.09^c
Tyrosine	2.06 ± 0.10^a	2.20 ± 0.02^b	2.42 ± 0.06^c	2.52 ± 0.02^d	2.08 ± 0.05^a	2.32 ± 0.08^c	2.27 ± 0.04^b	2.44 ± 0.09^c
Lysine	4.86 ± 0.32^a	5.43 ± 0.18^{bd}	5.36 ± 0.05^b	5.38 ± 0.13^{bd}	4.71 ± 0.12^a	5.45 ± 0.07^b	5.13 ± 0.08^c	5.53 ± 0.20^d
Histidine	1.31 ± 0.06^a	1.37 ± 0.03^a	1.53 ± 0.05^b	1.65 ± 0.04^c	1.35 ± 0.02^a	1.36 ± 0.05^a	1.47 ± 0.03^b	1.58 ± 0.07^c
Arginine	5.77 ± 0.30^a	6.08 ± 0.19^a	3.93 ± 0.08^b	4.03 ± 0.10^b	4.92 ± 0.24^c	4.47 ± 0.15^d	5.35 ± 0.14^c	5.77 ± 0.16^a
Σ EAA	28.52 ± 0.33^a	30.31 ± 0.58^b	33.00 ± 0.27^c	32.55 ± 0.88^c	27.83 ± 0.84^a	29.37 ± 0.74^b	30.28 ± 0.21^b	32.62 ± 0.93^c
Non-essential (NEAA)								
Aspartic acid	4.36 ± 0.23^a	5.10 ± 0.18^b	5.48 ± 0.06^c	5.90 ± 0.16^d	5.41 ± 0.56^c	4.96 ± 0.13^b	5.88 ± 0.40^d	6.33 ± 0.32^c
Serine	2.35 ± 0.13^a	2.79 ± 0.09^b	3.15 ± 0.08^c	3.15 ± 0.02^c	2.71 ± 0.12^b	2.62 ± 0.10^b	2.95 ± 0.03^d	3.18 ± 0.06^c
Glutamic acid	6.13 ± 0.10^a	7.02 ± 0.22^b	5.81 ± 0.18^c	7.88 ± 0.29^d	6.16 ± 0.36^a	7.14 ± 0.31^b	6.70 ± 0.15^c	7.21 ± 0.09^b
Glycine	2.70 ± 0.11^a	2.99 ± 0.09^b	1.97 ± 0.04^c	2.54 ± 0.11^d	2.92 ± 0.15^b	3.15 ± 0.09^e	3.18 ± 0.06^e	3.42 ± 0.08^f
Alanine	3.16 ± 0.09^a	3.37 ± 0.13^b	2.78 ± 0.06^c	3.42 ± 0.10^b	3.10 ± 0.09^a	3.31 ± 0.11^b	3.38 ± 0.05^b	3.64 ± 0.13^d
Cystine	tr	0.73 ± 0.04^a	0.70 ± 0.02^a	0.53 ± 0.03^b	0.70 ± 0.06^a	0.10 ± 0.16^c	0.77 ± 0.09^a	0.83 ± 0.11^a
Proline	3.27 ± 0.24^a	2.58 ± 0.05^b	2.27 ± 0.06^c	3.04 ± 0.13^a	3.01 ± 0.14^a	3.83 ± 0.26^d	3.27 ± 0.11^a	3.53 ± 0.16^d
Σ NEAA	21.96 ± 1.01^a	24.58 ± 0.71^b	22.16 ± 0.48^a	26.46 ± 0.31^c	24.02 ± 1.33^{bd}	25.10 ± 0.78^d	26.12 ± 0.59^d	28.13 ± 0.58^e
Σ TAA	50.49 ± 2.31^a	54.89 ± 1.30^b	55.16 ± 0.70^b	59.01 ± 0.78^c	51.86 ± 2.15^a	54.47 ± 1.51^b	56.41 ± 0.48^b	60.75 ± 1.29^c

Values are mean of triplicates $\pm$ SD. Different superscript letters within rows represent significant differences ($P < 0.05$); tr—trace.

Appendix Table 2. *Total (protein bound + free) amino acid composition (% dry weight) in the digestive gland*

Amino acids (% dw)	Illex coindetii				Todaropsis eblanae			
	Males		Females		Males		Females	
	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature
Essential (EAA)								
Chreonine	2.82 ± 0.02^a	2.20 ± 0.06^b	2.92 ± 0.10^a	2.74 ± 0.08^a	2.78 ± 0.29^{ac}	2.19 ± 0.06^b	2.52 ± 0.08^c	2.34 ± 0.07^d
Methionine	1.84 ± 0.07^a	1.40 ± 0.03^b	1.91 ± 0.07^a	1.79 ± 0.07^a	1.83 ± 0.18^{ac}	1.42 ± 0.01^b	1.64 ± 0.01^c	1.52 ± 0.01^d
Isoleucine	3.03 ± 0.04^a	2.58 ± 0.06^b	3.23 ± 0.12^c	3.03 ± 0.15^{ac}	3.07 ± 0.34^{acd}	2.45 ± 0.05^d	2.82 ± 0.07^e	2.62 ± 0.07^f
Leucine	4.41 ± 0.09^a	3.19 ± 0.08^b	4.87 ± 0.17^c	4.57 ± 0.19^{ac}	4.63 ± 0.51^{abc}	3.59 ± 0.03^d	4.13 ± 0.02^c	3.84 ± 0.01^f
Valine	3.38 ± 0.08^a	2.44 ± 0.05^b	3.50 ± 0.13^a	3.29 ± 0.13^a	3.33 ± 0.37^a	2.50 ± 0.04^b	2.88 ± 0.05^c	2.68 ± 0.05^d
Phenylalanine	3.44 ± 0.07^a	2.40 ± 0.06^b	4.19 ± 0.15^c	3.93 ± 0.16^c	4.00 ± 0.41^c	2.92 ± 0.33^b	3.36 ± 0.39^a	3.13 ± 0.37^a
Tyrosine	2.83 ± 0.16^a	1.77 ± 0.04^b	3.00 ± 0.11^a	2.82 ± 0.08^a	2.81 ± 0.38^a	1.88 ± 0.02^c	2.17 ± 0.02^d	2.02 ± 0.02^e
Lysine	5.39 ± 0.14^a	3.86 ± 0.09^b	6.09 ± 0.22^c	5.72 ± 0.14^d	5.75 ± 0.71^d	4.61 ± 0.07^e	5.30 ± 0.07^a	4.93 ± 0.06^f
Histidine	1.78 ± 0.03^a	1.24 ± 0.04^{bd}	0.15 ± 0.01^c	0.14 ± 0.01^c	0.77 ± 0.87^{bd}	1.17 ± 0.05^b	1.34 ± 0.06^d	1.25 ± 0.05^{bd}
Arginine	3.69 ± 0.10^a	2.37 ± 0.07^b	4.07 ± 0.15^c	3.82 ± 0.16^{ac}	3.89 ± 0.40^{ac}	2.80 ± 0.04^d	3.22 ± 0.05^e	2.99 ± 0.05^f
Σ EAA	32.59 ± 0.79^a	23.47 ± 0.56^b	33.93 ± 1.21^{ad}	31.87 ± 1.30^{ad}	32.86 ± 2.73^{ad}	25.53 ± 0.45^c	29.39 ± 0.58^d	27.33 ± 0.58^c
Non-essential (NEAA)								
Aspartic acid	6.15 ± 0.12^a	4.19 ± 0.14^b	6.43 ± 0.23^a	6.04 ± 0.25^a	5.64 ± 0.07^b	4.73 ± 0.02^c	5.47 ± 0.03^d	5.07 ± 0.03^e
Serine	2.65 ± 0.03^a	2.08 ± 0.05^b	2.80 ± 0.10^a	2.63 ± 0.11^a	2.70 ± 0.25^a	2.07 ± 0.01^b	2.39 ± 0.02^c	2.22 ± 0.02^d
Glutamic acid	7.30 ± 0.15^a	5.33 ± 0.12^b	8.10 ± 0.29^c	7.61 ± 0.31^a	6.30 ± 0.01^d	7.03 ± 0.02^e	8.09 ± 0.04^c	7.52 ± 0.05^a
Glycine	3.10 ± 0.12^a	1.97 ± 0.06^b	3.72 ± 0.13^c	3.49 ± 0.14^d	3.57 ± 0.34^{cd}	2.57 ± 0.03^e	2.96 ± 0.03^f	2.75 ± 0.04^g
Alanine	3.34 ± 0.07^a	2.36 ± 0.09^b	3.62 ± 0.13^c	3.40 ± 0.17^{ac}	3.49 ± 0.32^{ac}	2.89 ± 0.04^d	3.33 ± 0.06^a	3.09 ± 0.06^a
Cystine	tr	1.05 ± 0.03	tr	tr	tr	tr	tr	tr
Proline	2.92 ± 0.19^a	1.85 ± 0.10^b	3.78 ± 0.14^{ce}	3.55 ± 0.11^c	2.96 ± 0.11^a	3.61 ± 0.07^c	4.16 ± 0.08^d	3.87 ± 0.07^e
Σ NEAA	25.46 ± 0.67^a	18.83 ± 0.51^b	28.46 ± 1.02^a	26.73 ± 0.98^a	24.66 ± 0.73^{ac}	22.91 ± 0.06^d	26.37 ± 0.12^c	24.52 ± 0.15^{ac}
Σ TAA	58.05 ± 1.46^a	42.30 ± 1.02^b	59.74 ± 2.14^a	56.11 ± 2.21^{ad}	57.52 ± 3.46^{ad}	48.44 ± 0.51^c	55.76 ± 0.70^d	51.85 ± 0.83^c

Values are mean of triplicates $\pm$ SD. Different superscript letters within rows represent significant differences ($P < 0.05$); tr—trace.

R. ROSA *ET AL.*

Appendix Table 3. *Total (protein bound + free) amino acid composition (% dry weight) in the muscle*

Amino acids (% dw)	*Illex coindetii*				*Todaropsis eblanae*			
	Males		Females		Males		Females	
	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature
Essential (EAA)								
Threonine	3.07 ± 0.10ᵃ	2.92 ± 0.04ᵃ	2.99 ± 0.13ᵃ	3.04 ± 0.14ᵃ	2.96 ± 0.03ᵃ	2.75 ± 0.08ᵇ	2.69 ± 0.39ᵃᵇ	2.88 ± 0.06ᵃ
Methionine	2.30 ± 0.09ᵃ	2.19 ± 0.05ᵇ	2.24 ± 0.08ᵃᵇ	2.28 ± 0.09ᵃᵇ	1.17 ± 1.50ᶜᵇ	0.99 ± 1.28ᵃᵇ	1.97 ± 0.25ᶜ	2.10 ± 0.13ᵇ
Isoleucine	3.22 ± 0.06ᵃ	3.07 ± 0.01ᵇ	3.14 ± 0.18ᵃᵇ	3.20 ± 0.19ᵃᵇ	2.90 ± 0.05ᶜ	2.83 ± 0.12ᶜ	2.82 ± 0.48ᶜ	3.01 ± 0.10ᵇᶜ
Leucine	5.65 ± 0.18ᵃ	5.38 ± 0.08ᵇ	5.51 ± 0.24ᵃ	5.61 ± 0.27ᵃ	5.26 ± 0.06ᵇ	4.86 ± 0.10ᶜ	4.83 ± 0.15ᶜ	5.15 ± 0.25ᵇ
Valine	2.97 ± 0.07ᵃ	2.82 ± 0.02ᵇ	2.89 ± 0.15ᵃᵇ	2.94 ± 0.16ᵃᵇ	2.88 ± 0.04ᵃᵇ	2.80 ± 0.01ᵇ	2.62 ± 0.12ᵇ	2.80 ± 0.03ᵇ
Phenylalanine	3.26 ± 0.07ᵃ	3.11 ± 0.01ᵇ	3.18 ± 0.17ᵃᵇ	3.24 ± 0.19ᵃᵇ	3.11 ± 0.03ᵇ	3.25 ± 0.34ᵃᵇ	2.81 ± 0.54ᵃᵇ	3.02 ± 0.19ᵃᵇ
Tyrosine	2.52 ± 0.05ᵃ	2.40 ± 0.01ᵇ	2.46 ± 0.14ᵃᵇ	2.51 ± 0.15ᵃᵇ	2.44 ± 0.04ᵇ	2.55 ± 0.16ᵃᵇ	2.28 ± 0.29ᵃᵇ	2.45 ± 0.05ᵇ
Lysine	5.88 ± 0.14ᵃ	5.60 ± 0.03ᵇ	5.73 ± 0.30ᵃᵇ	5.84 ± 0.33ᵃᵇ	5.57 ± 0.06ᵇ	5.34 ± 0.22ᵇ	5.17 ± 0.90ᵃᵇ	5.52 ± 0.26ᵃᵇ
Histidine	1.71 ± 0.04ᵃ	1.63 ± 0.01ᵇ	1.67 ± 0.09ᵃᵇ	1.70 ± 0.10ᵃᵇ	2.60 ± 0.02ᶜ	1.14 ± 0.20ᵈ	1.52 ± 0.28ᵉ	1.62 ± 0.09ᵉ
Arginine	5.65 ± 0.13ᵃ	5.38 ± 0.03ᵇ	5.51 ± 0.29ᵃᵇ	5.61 ± 0.31ᵃᵇ	4.73 ± 0.07ᶜ	5.36 ± 0.41ᵃᵇ	4.72 ± 0.74ᵇᶜ	5.04 ± 0.15ᵇᶜ
Σ EAA	36.23 ± 0.92ᵃ	34.50 ± 1.29ᵃᵇ	35.31 ± 1.77ᵃᵇ	35.96 ± 1.93ᵃᵇ	33.60 ± 1.09ᵇᶜ	31.87 ± 1.35ᶜ	31.43 ± 2.20ᶜ	33.57 ± 1.30ᵇᶜ
Non-essential (NEAA)								
Aspartic acid	7.11 ± 0.24ᵃ	6.77 ± 0.11ᵇ	6.93 ± 0.29ᵃᵇ	7.05 ± 0.32ᵃᵇ	6.53 ± 0.09ᶜ	6.39 ± 0.21ᶜᵈ	6.01 ± 0.20ᵈ	6.41 ± 0.28ᶜᵈ
Serine	3.11 ± 0.15ᵃ	2.96 ± 0.09ᵃᵇ	3.02 ± 0.09ᵃᵇ	3.08 ± 0.10ᵃᵇ	2.87 ± 0.02ᵇ	2.83 ± 0.10ᵃᵇ	2.68 ± 0.41ᵃᵇ	2.87 ± 0.07ᵇ
Glutamic acid	10.43 ± 0.38ᵃ	9.94 ± 0.19ᵃ	10.17 ± 0.40ᵃ	10.35 ± 0.44ᵃ	10.03 ± 0.04ᵃ	9.01 ± 0.43ᵇ	8.96 ± 0.53ᵇ	9.57 ± 0.39ᵃᵇ
Glycine	3.51 ± 0.12ᵃ	3.34 ± 0.05ᵇ	3.42 ± 0.14ᵃᵇ	3.48 ± 0.16ᵃᵇ	3.96 ± 0.06ᶜ	4.56 ± 0.21ᵈ	3.12 ± 0.25ᵇ	3.32 ± 0.17ᵃᵇ
Alanine	4.26 ± 0.15ᵃ	4.06 ± 0.07ᵃ	4.15 ± 0.17ᵃ	4.23 ± 0.19ᵃ	4.49 ± 0.01ᵇ	4.05 ± 0.05ᵃ	4.30 ± 0.03ᵇ	4.64 ± 0.62ᵃᵇ
Cystine	0.51 ± 0.02ᵃ	0.49 ± 0.01ᵃ	0.50 ± 0.02ᵃ	0.51 ± 0.02ᵃ	0.12 ± 0.17ᵇ	0.12 ± 0.17ᵇ	0.64 ± 0.15ᵃ	0.69 ± 0.08ᶜ
Proline	2.93 ± 0.13ᵃ	2.79 ± 0.08ᵃ	2.86 ± 0.09ᵃ	2.91 ± 0.10ᵃ	4.84 ± 0.39ᵇ	3.47 ± 0.21ᶜ	3.31 ± 0.39ᵃ	3.59 ± 0.87ᵃᶜ
Σ NEAA	31.86 ± 0.19ᵃ	30.34 ± 0.61ᵇ	31.04 ± 1.19ᵃᵇ	31.61 ± 1.33ᵃᵇ	32.85 ± 0.76ᵇ	30.35 ± 1.28ᵃᵇ	29.02 ± 2.12ᵃᵇ	31.10 ± 0.51ᵃᵇ
Σ TAA	68.09 ± 2.11ᵃ	64.85 ± 0.90ᵇᶜ	66.36 ± 2.96ᵃᵇ	67.57 ± 3.26ᵃᵇ	66.45 ± 0.33ᵃ	62.22 ± 1.07ᵇ	60.45 ± 2.41ᵇᶜ	64.68 ± 0.78ᶜ

Values are mean of triplicates ± SD. Different superscript letters within rows represent significant differences ($P < 0.05$).

Appendix Table 4. *Fatty acid composition (µg/mg dry weight) in the gonad*

Fatty acids (µg/mg DW)	Illex coindetii				Todaropsis eblanae			
	Males		Females		Males		Females	
	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature
12:0	0.03 ± 0.01^a	0.01 ± 0.00^b	0.04 ± 0.00^a	0.07 ± 0.00^c	0.01 ± 0.01^b	0.02 ± 0.00^b	0.03 ± 0.01^a	0.04 ± 0.00^a
14:0	0.97 ± 0.14^a	1.30 ± 0.12^b	1.90 ± 0.05^c	4.26 ± 0.31^d	0.80 ± 0.13^a	1.26 ± 0.25^b	2.17 ± 0.22^c	4.86 ± 0.58^d
15:0	0.14 ± 0.02^a	0.27 ± 0.01^b	0.33 ± 0.02^c	0.38 ± 0.02^d	0.10 ± 0.01^e	0.22 ± 0.03^b	0.12 ± 0.01^a	0.52 ± 0.07^f
16:0	10.72 ± 1.31^a	13.35 ± 0.64^b	24.50 ± 0.74^c	40.67 ± 2.60^d	10.42 ± 0.74^a	14.10 ± 1.59^b	18.58 ± 0.38^c	50.60 ± 6.78^f
17:0	0.53 ± 0.05^a	0.64 ± 0.01^b	1.12 ± 0.03^c	1.02 ± 0.06^d	0.56 ± 0.03^a	0.90 ± 0.11^c	0.47 ± 0.00^f	1.19 ± 0.16^{cd}
18:0	2.39 ± 0.20^a	3.08 ± 0.15^b	5.34 ± 0.14^c	7.77 ± 0.48^d	5.07 ± 0.15^c	5.88 ± 0.41^c	6.03 ± 0.31^e	12.82 ± 1.63^f
19:0	0.03 ± 0.00^a	0.02 ± 0.00^b	0.04 ± 0.03^{ab}	0.13 ± 0.00^c	0.04 ± 0.01^a	0.04 ± 0.02^{abd}	0.06 ± 0.01^d	0.15 ± 0.02^c
20:0	0.07 ± 0.01^a	0.07 ± 0.01^a	0.14 ± 0.01^b	0.17 ± 0.01^c	0.08 ± 0.00^a	0.14 ± 0.01^b	0.14 ± 0.04^b	0.17 ± 0.02^c
22:0	0.03 ± 0.01^a	0.02 ± 0.00^a	0.07 ± 0.01^b	0.05 ± 0.01^c	0.04 ± 0.01^{ac}	0.03 ± 0.01^a	0.10 ± 0.03^b	0.14 ± 0.05^d
Σ Saturated	14.90 ± 1.70^a	18.72 ± 0.93^b	33.47 ± 0.95^c	54.53 ± 3.46^d	17.12 ± 1.07^b	22.58 ± 2.36^e	27.70 ± 0.94^f	70.48 ± 9.26^g
Iso 14:0	0.09 ± 0.10^{ab}	0.02 ± 0.00^a	0.01 ± 0.01^a	0.06 ± 0.08^b	0.02 ± 0.00^a	0.02 ± 0.01^a	0.01 ± 0.01^a	0.03 ± 0.01^a
Anteiso 14:0	0.03 ± 0.01^a	0.01 ± 0.00^b	0.01 ± 0.00^b	0.01 ± 0.00^b	0.00 ± 0.00^c	0.00 ± 0.00^c	0.01 ± 0.01^a	0.03 ± 0.01^a
Iso 16:0	0.04 ± 0.02^a	0.10 ± 0.01^b	0.14 ± 0.00^c	0.21 ± 0.02^d	0.10 ± 0.01^b	0.10 ± 0.01^b	0.00 ± 0.00^c	0.00 ± 0.00^c
Anteiso 16:0	0.07 ± 0.01^a	0.11 ± 0.01^{bc}	0.12 ± 0.01^b	0.10 ± 0.01^c	0.07 ± 0.01^a	0.11 ± 0.01^{bc}	0.07 ± 0.01^e	0.30 ± 0.04^f
Σ Branched	0.23 ± 0.01^a	0.24 ± 0.01^a	0.28 ± 0.01^b	0.39 ± 0.12^c	0.18 ± 0.01^d	0.23 ± 0.04^a	0.12 ± 0.02^c	0.39 ± 0.03^c
16:1n-7	0.21 ± 0.02^a	0.24 ± 0.02^{ad}	0.31 ± 0.02^b	1.12 ± 0.11^c	0.26 ± 0.02^d	0.25 ± 0.02^{ad}	0.41 ± 0.01^e	1.37 ± 0.19^f
17:1n-8	0.03 ± 0.03^a	0.08 ± 0.00^b	0.18 ± 0.01^c	0.18 ± 0.11^c	0.52 ± 0.02^d	0.09 ± 0.00^b	0.18 ± 0.02^c	0.46 ± 0.06^d
18:1n-9	1.53 ± 0.10^a	1.89 ± 0.10^b	7.62 ± 0.31^c	11.27 ± 0.71^d	2.45 ± 0.07^e	2.03 ± 0.21^b	9.58 ± 0.89^f	20.17 ± 2.56^g
18:1n-7	0.69 ± 0.05^a	0.85 ± 0.05^b	1.13 ± 0.03^c	2.50 ± 0.18^d	1.35 ± 0.03^e	1.44 ± 0.11^e	1.91 ± 0.01^f	5.60 ± 0.55^g
20:1n-9	0.24 ± 0.04^a	0.36 ± 0.09^b	0.01 ± 0.00^c	0.01 ± 0.00^c	0.01 ± 0.00^c	0.00 ± 0.00^d	0.00 ± 0.00^d	0.01 ± 0.00^c
20:1n-7	4.21 ± 0.21^a	4.30 ± 0.15^a	7.46 ± 0.22^b	11.51 ± 0.48^c	7.82 ± 0.10^b	10.43 ± 0.97^c	10.68 ± 2.70^c	20.78 ± 2.71^d
22:1n-11	0.05 ± 0.02^a	0.01 ± 0.00^b	0.07 ± 0.01^a	0.37 ± 0.06^c	0.25 ± 0.00^d	0.05 ± 0.01^a	0.14 ± 0.01^c	0.24 ± 0.03^d
22:1n-9	0.49 ± 0.03^a	0.43 ± 0.01^b	0.35 ± 0.03^c	0.34 ± 0.02^c	0.76 ± 0.01^d	1.02 ± 0.09^e	0.51 ± 0.07^a	0.52 ± 0.05^a
Σ Monounsaturated	7.45 ± 0.41^a	8.15 ± 0.26^b	17.14 ± 0.53^c	27.29 ± 1.60^d	13.42 ± 0.25^e	15.31 ± 1.36^e	23.42 ± 3.69^d	49.15 ± 6.01^f
16:4n-3	0.45 ± 0.01^a	0.48 ± 0.03^a	0.54 ± 0.02^b	0.52 ± 0.11^{ab}	0.51 ± 0.01^b	0.85 ± 0.11^c	0.34 ± 0.02^d	0.48 ± 0.05^a
18:2n-6	0.04 ± 0.01^a	0.08 ± 0.00^b	0.20 ± 0.02^c	0.35 ± 0.01^d	0.09 ± 0.01^b	0.11 ± 0.10^{abc}	0.16 ± 0.02^c	0.49 ± 0.06^c
18:3n-6	0.03 ± 0.01^a	0.05 ± 0.00^b	0.09 ± 0.02^c	0.13 ± 0.01^d	0.04 ± 0.03^{abc}	0.03 ± 0.01^a	0.07 ± 0.00^c	0.49 ± 0.06^c
18:3n-3	0.05 ± 0.03^{ab}	0.03 ± 0.00^a	0.08 ± 0.01^b	0.20 ± 0.00^c	0.12 ± 0.00^d	0.36 ± 0.14^e	0.11 ± 0.02^d	0.39 ± 0.04^d
18:4n-3	1.06 ± 0.06^a	1.27 ± 0.31^{ab}	0.73 ± 0.10^c	1.05 ± 0.40^a	1.27 ± 0.02^b	1.92 ± 0.63^c	0.85 ± 0.14^d	0.06 ± 0.01^b
20:2n-6	0.11 ± 0.04^{bc}	0.21 ± 0.02^b	0.27 ± 0.02^c	0.49 ± 0.08^d	0.16 ± 0.01^e	0.13 ± 0.00^a	0.40 ± 0.01^f	0.88 ± 0.14^d
20:4n-6	0.54 ± 0.02^a	1.18 ± 0.10^b	1.36 ± 0.05^c	2.66 ± 0.13^d	1.38 ± 0.00^c	1.45 ± 0.13^c	2.85 ± 0.01^d	1.00 ± 0.07^g
20:3n-3	0.11 ± 0.02^a	0.10 ± 0.02^a	0.15 ± 0.01^b	0.28 ± 0.02^c	0.09 ± 0.00^a	0.14 ± 0.00^b	0.23 ± 0.10^{abc}	4.81 ± 0.66^e
20:4n-3	0.07 ± 0.03^{ab}	0.04 ± 0.00^d	0.12 ± 0.02^b	0.38 ± 0.01^c	0.08 ± 0.00^b	0.05 ± 0.00^a	0.22 ± 0.00^d	0.72 ± 0.07^e
20:5n-3	10.07 ± 0.63^a	10.67 ± 0.48^a	13.06 ± 0.65^b	19.31 ± 0.94^c	15.36 ± 0.10^d	23.35 ± 2.13^c	13.85 ± 0.90^b	28.83 ± 1.85^c
22:4n-6	0.09 ± 0.01^a	0.04 ± 0.01^b	0.09 ± 0.01^a	0.38 ± 0.03^c	0.06 ± 0.01^d	0.06 ± 0.00^d	0.15 ± 0.01^e	0.41 ± 0.06^c
22:5n-6	0.07 ± 0.01^a	0.17 ± 0.03^b	0.40 ± 0.05^c	0.82 ± 0.11^d	0.23 ± 0.05^b	0.19 ± 0.01^b	0.43 ± 0.02^c	1.34 ± 0.27^e
22:5n-3	0.13 ± 0.01^a	0.17 ± 0.06^a	0.01 ± 0.00^b	1.54 ± 0.07^c	0.31 ± 0.04^d	0.38 ± 0.03^d	1.12 ± 0.21^c	2.16 ± 0.55^c
22:6n-3	9.58 ± 0.64^a	11.85 ± 0.54^b	26.45 ± 1.64^c	40.06 ± 2.33^d	14.65 ± 0.38^e	18.83 ± 1.82^f	23.03 ± 2.14^c	60.27 ± 8.39^g
Σ Polyunsaturated	22.39 ± 1.55^a	26.34 ± 1.52^b	43.54 ± 2.15^c	68.15 ± 3.91^d	34.36 ± 0.29^e	47.84 ± 4.83^c	43.82 ± 3.17^c	102.37 ± 12.67^f
Σ Total FA	44.97 ± 3.71^a	53.42 ± 2.66^b	94.43 ± 3.62^c	150.36 ± 9.07^d	65.08 ± 1.05^e	85.97 ± 8.49^c	95.06 ± 7.75^c	222.38 ± 28.99^f

Different superscript letters within rows represent significant differences ($P < 0.05$).

Appendix Table 5. *Fatty acid composition (μg/mg dry weight) in the digestive gland*

Fatty acids (μg/mg DW)	*Illex coindetii*				*Todaropsis eblanae*			
	Males		Females		Males		Females	
	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature
12:0	0.01 ± 0.00^a	0.12 ± 0.02^b	0.03 ± 0.02^a	0.51 ± 0.03^c	0.15 ± 0.00^b	0.14 ± 0.01^b	0.10 ± 0.02^b	0.12 ± 0.02^b
14:0	6.22 ± 0.06^a	5.89 ± 0.05^b	9.79 ± 0.29^c	5.59 ± 0.05^d	4.22 ± 0.06^e	7.80 ± 0.29^f	4.18 ± 0.57^e	7.93 ± 0.55^f
15:0	1.37 ± 0.04^a	0.86 ± 0.13^b	2.13 ± 0.19^c	2.88 ± 0.00^d	0.63 ± 0.04^e	1.03 ± 0.05^f	0.47 ± 0.06^g	1.24 ± 0.17^{af}
16:0	34.64 ± 0.35^a	33.00 ± 3.93^{ac}	46.26 ± 3.08^b	31.66 ± 0.45^c	24.73 ± 0.35^d	45.67 ± 2.31^b	25.13 ± 2.59^d	34.99 ± 4.98^{ac}
17:0	2.10 ± 0.02^a	1.53 ± 0.14^{bg}	3.38 ± 0.15^c	4.23 ± 0.11^d	1.11 ± 0.02^e	1.78 ± 0.18^b	0.95 ± 0.06^f	1.30 ± 0.29^g
18:0	10.71 ± 0.02^a	10.68 ± 0.81^a	11.54 ± 0.36^a	19.50 ± 1.08^b	16.26 ± 0.02^c	18.00 ± 1.52^b	13.95 ± 0.90^d	16.21 ± 2.45^{bc}
19:0	0.33 ± 0.02^a	0.19 ± 0.01^b	0.18 ± 0.01^b	1.05 ± 0.08^c	0.38 ± 0.01^d	0.04 ± 0.01^e	0.29 ± 0.04^a	0.09 ± 0.01^f
20:0	0.61 ± 0.03^a	0.42 ± 0.01^b	0.62 ± 0.04^a	1.49 ± 0.07^c	0.43 ± 0.03^b	0.74 ± 0.07^d	0.47 ± 0.05^b	0.80 ± 0.12^d
22:0	0.34 ± 0.02^a	0.20 ± 0.02^{bc}	0.48 ± 0.09^c	0.73 ± 0.01^d	0.17 ± 0.01^b	0.53 ± 0.26^{cd}	0.25 ± 0.04^c	0.37 ± 0.00^t
Σ Saturated	56.33 ± 0.44^a	52.93 ± 1.10^b	74.41 ± 1.22^c	67.65 ± 1.82^d	48.08 ± 0.44^e	75.74 ± 1.14^c	45.80 ± 2.95^e	63.05 ± 1.58^d
Iso 14:0	0.37 ± 0.02^a	0.23 ± 0.04^b	0.67 ± 0.06^c	2.10 ± 0.04^d	0.47 ± 0.02^e	0.31 ± 0.01^f	0.14 ± 0.04^g	0.46 ± 0.09^e
Anteiso 14:0	0.15 ± 0.01^a	0.10 ± 0.02^b	0.35 ± 0.04^c	1.17 ± 0.05^d	0.25 ± 0.01^e	0.05 ± 0.01^f	0.06 ± 0.02^f	0.08 ± 0.02^{bf}
Iso 16:0	0.95 ± 0.01^a	0.99 ± 0.14^a	1.39 ± 0.09^b	3.47 ± 0.08^c	0.62 ± 0.01^d	1.44 ± 0.07^b	0.53 ± 0.10^d	0.98 ± 0.15^a
Anteiso 16:0	0.38 ± 0.02^a	0.25 ± 0.02^b	0.52 ± 0.02^c	1.87 ± 0.06^d	0.32 ± 0.02^a	0.35 ± 0.02^a	0.16 ± 0.06^b	0.40 ± 0.10^a
Σ Branched	1.86 ± 0.03^a	1.57 ± 0.22^b	2.93 ± 0.23^c	8.60 ± 0.05^d	1.65 ± 0.03^b	2.14 ± 0.09^c	0.90 ± 0.18^f	1.92 ± 0.28^a
16:1n-7	5.51 ± 0.02^a	4.54 ± 0.20^b	4.01 ± 0.21^c	14.59 ± 0.79^d	7.26 ± 0.02^e	7.68 ± 0.57^e	4.51 ± 0.34^b	9.57 ± 1.52^f
17:1n-8	1.28 ± 0.02^a	0.77 ± 0.05^b	1.60 ± 0.17^c	2.88 ± 0.14^d	1.32 ± 0.02^a	1.21 ± 0.09^a	0.61 ± 0.06^e	1.70 ± 0.26^c
18:1n-9	30.92 ± 0.35^a	27.21 ± 2.01^b	32.11 ± 1.56^a	26.46 ± 1.25^b	43.33 ± 0.35^c	44.65 ± 3.56^c	22.20 ± 1.31^d	60.93 ± 9.92^e
18:1n-7	6.38 ± 0.52^a	5.16 ± 0.28^b	6.35 ± 0.36^a	18.84 ± 0.17^c	7.40 ± 0.52^d	8.07 ± 0.98^d	4.64 ± 0.17^e	11.48 ± 1.62^t
20:1n-9	0.69 ± 0.02^a	1.10 ± 0.00^b	0.73 ± 0.18^a	6.27 ± 0.18^c	0.01 ± 0.00^d	0.01 ± 0.00^d	2.65 ± 0.65^e	0.01 ± 0.00^d
20:1n-7	4.70 ± 0.06^a	12.59 ± 1.48^b	4.67 ± 0.12^a	6.72 ± 0.06^c	17.77 ± 0.06^d	22.84 ± 2.52^e	13.40 ± 0.95^b	21.86 ± 3.34^e
22:1n-11	1.37 ± 0.01^a	6.43 ± 0.70^b	1.50 ± 0.10^a	0.97 ± 0.11^c	4.65 ± 0.01^d	14.03 ± 1.26^e	6.28 ± 0.14^b	5.95 ± 0.80^b
22:1n-9	0.71 ± 0.02^a	1.11 ± 0.12^b	0.59 ± 0.02^c	1.41 ± 0.03^d	1.22 ± 0.02^b	1.91 ± 0.17^c	1.27 ± 0.12^b	2.11 ± 0.31^e
Σ Monounsaturated	51.56 ± 0.97^a	58.92 ± 2.15^b	51.55 ± 2.72^a	78.15 ± 0.41^c	82.95 ± 0.97^d	100.39 ± 8.88^e	55.66 ± 2.21^b	113.61 ± 17.76^c
16:4n-3	0.54 ± 0.01^a	0.59 ± 0.00^b	0.65 ± 0.01^c	2.96 ± 0.23^d	1.13 ± 0.01^e	0.77 ± 0.12^{bc}	1.14 ± 0.10^e	0.65 ± 0.13^c
18:2n-6	2.79 ± 0.07^a	1.39 ± 0.05^b	4.49 ± 0.18^c	3.00 ± 0.06^d	3.03 ± 0.07^d	2.46 ± 0.28^a	1.31 ± 0.05^b	4.15 ± 0.91^c
18:3n-6	0.68 ± 0.02^a	0.40 ± 0.02^b	0.87 ± 0.04^c	1.09 ± 0.24^c	0.56 ± 0.02^d	0.67 ± 0.07^a	0.24 ± 0.02^e	0.79 ± 0.10^a
18:3n-3	1.80 ± 0.07^a	0.82 ± 0.03^b	2.15 ± 0.01^c	0.56 ± 0.01^d	1.21 ± 0.07^e	1.20 ± 0.07^e	0.48 ± 0.07^d	1.86 ± 0.27^a
18:4n-3	1.21 ± 0.06^a	0.63 ± 0.02^b	1.48 ± 0.09^a	1.07 ± 0.13^d	1.55 ± 0.06^a	2.85 ± 0.50^e	4.19 ± 0.31^f	3.03 ± 0.65^g
20:2n-6	1.08 ± 0.05^a	0.89 ± 0.09^b	1.57 ± 0.04^c	4.84 ± 0.06^d	1.77 ± 0.05^e	1.51 ± 0.14^c	1.04 ± 0.08^a	2.37 ± 0.34^f
20:4n-6	3.23 ± 0.08^a	3.69 ± 0.15^b	3.56 ± 0.05^b	14.24 ± 0.01^c	4.53 ± 0.08^d	4.80 ± 0.52^d	6.20 ± 0.47^e	5.57 ± 0.26^f
20:3n-3	0.50 ± 0.02^a	0.51 ± 0.02^a	0.45 ± 0.01^c	0.68 ± 0.03^c	0.45 ± 0.02^b	0.49 ± 0.20^{ab}	0.29 ± 0.00^d	0.89 ± 0.21^e
20:4n-3	1.31 ± 0.05^a	0.84 ± 0.04^b	0.94 ± 0.01^c	1.19 ± 0.01^d	0.98 ± 0.05^c	1.19 ± 0.13^d	0.73 ± 0.08^b	1.84 ± 0.25^e
20:5n-3	14.48 ± 0.47^a	12.21 ± 0.86^b	22.10 ± 0.08^c	35.46 ± 0.37^d	13.41 ± 0.47^a	15.24 ± 1.54^a	20.31 ± 1.89^c	20.23 ± 2.42^c
22:4n-6	0.38 ± 0.11^a	0.01 ± 0.00^b	0.35 ± 0.01^a	1.66 ± 1.08^c	0.36 ± 0.11^a	0.21 ± 0.10^d	0.37 ± 0.07^a	0.49 ± 0.03^a
22:5n-6	0.77 ± 0.05^a	0.97 ± 0.09^b	0.94 ± 0.00^b	1.64 ± 0.04^c	0.95 ± 0.05^b	0.99 ± 0.11^b	1.10 ± 0.15^b	1.71 ± 0.18^c
22:5n-3	1.22 ± 0.00^a	2.14 ± 0.23^b	1.14 ± 0.00^c	4.38 ± 0.13^d	0.75 ± 0.00^e	2.70 ± 0.28^f	2.80 ± 0.27^f	3.81 ± 0.11^g
22:6n-3	26.40 ± 0.34^a	34.13 ± 1.18^b	39.32 ± 0.30^c	29.13 ± 0.76^d	32.79 ± 0.34^b	34.28 ± 3.56^b	45.94 ± 2.73^c	53.66 ± 4.66^f
Σ Polyunsaturated	56.38 ± 1.38^a	59.22 ± 1.53^b	80.00 ± 0.11^c	101.90 ± 2.09^d	63.46 ± 1.38^c	69.38 ± 6.68^c	86.17 ± 5.32^c	101.05 ± 13.01^d
Σ Total FA	166.13 ± 2.82^a	172.64 ± 15.99^b	208.89 ± 8.05^c	256.30 ± 1.63^d	196.15 ± 2.82^e	247.64 ± 18.00^d	188.53 ± 11.29^c	279.64 ± 10.64^f

Different superscript letters within rows represent significant differences ($P < 0.05$).

Appendix Table 6. *Fatty acid composition (μg/mg dry weight) in the muscle*

Fatty acids (μg/mg DW)	*Illex coindetii*				*Todaropsis eblanae*			
	Males		Females		Males		Females	
	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature
12:0	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^c	0.01 ± 0.00^a	0.00 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a
14:0	0.45 ± 0.02^a	0.25 ± 0.01^b	0.73 ± 0.20^{cd}	0.59 ± 0.06^c	0.66 ± 0.05^c	0.96 ± 0.04^d	0.98 ± 0.07^d	1.22 ± 0.04^e
15:0	0.17 ± 0.01^a	0.17 ± 0.00^a	0.21 ± 0.06^{ab}	0.17 ± 0.02^a	0.21 ± 0.02^b	0.23 ± 0.01^b	0.29 ± 0.02^c	0.25 ± 0.01^d
16:0	11.64 ± 0.64^a	8.67 ± 0.10^b	11.57 ± 0.99^a	10.63 ± 0.89^a	11.07 ± 0.59^a	11.84 ± 0.12^a	12.78 ± 0.71^a	12.07 ± 0.38^a
17:0	0.42 ± 0.04^a	0.82 ± 0.04^b	0.47 ± 0.03^{ab}	0.44 ± 0.04^a	0.49 ± 0.02^b	0.43 ± 0.01^a	0.50 ± 0.03^b	0.45 ± 0.02^a
18:0	2.72 ± 0.07^a	3.01 ± 0.10^b	2.66 ± 0.18^{ab}	2.90 ± 0.17^{ab}	2.66 ± 0.02^a	3.22 ± 0.04^c	2.03 ± 0.08^d	3.53 ± 0.13^e
19:0	0.02 ± 0.00^a	0.02 ± 0.01^a	0.02 ± 0.01^a	0.01 ± 0.01^a	0.02 ± 0.01^a	0.04 ± 0.02^b	0.03 ± 0.00^{ab}	0.06 ± 0.01^b
20:0	0.06 ± 0.02^{ab}	0.04 ± 0.01^b	0.06 ± 0.01^a	0.07 ± 0.00^{ac}	0.08 ± 0.00^c	0.07 ± 0.01^a	0.05 ± 0.00^{ab}	0.08 ± 0.01^c
22:0	0.02 ± 0.01^a	0.03 ± 0.00^a	0.07 ± 0.01^b	0.06 ± 0.02^b	0.03 ± 0.00^a	0.03 ± 0.00^a	0.06 ± 0.01^b	0.05 ± 0.04^{ab}
Σ Saturated	15.51 ± 0.99^a	13.02 ± 0.26^b	15.80 ± 0.82^a	14.88 ± 1.19^{ab}	15.21 ± 0.70^a	16.83 ± 0.16^{ac}	16.73 ± 0.90^{ac}	17.72 ± 0.60^c
Iso 14:0	0.01 ± 0.00^a	0.02 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.02 ± 0.01^a	0.02 ± 0.00^a	0.01 ± 0.00^a	0.02 ± 0.00^a
Anteiso 14:0	0.01 ± 0.00^a	0.02 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a
Iso 16:0	0.08 ± 0.01^a	0.08 ± 0.01^a	0.08 ± 0.01^a	0.08 ± 0.01^a	0.10 ± 0.00^b	0.08 ± 0.00^a	0.07 ± 0.00^a	0.10 ± 0.01^b
Anteiso 16:0	0.07 ± 0.01^a	0.16 ± 0.10^b	0.08 ± 0.02^a	0.07 ± 0.00^a	0.08 ± 0.03^b	0.08 ± 0.01^a	0.10 ± 0.00^c	0.08 ± 0.01^a
Σ Branched	0.17 ± 0.01^a	0.28 ± 0.11^b	0.17 ± 0.01^a	0.16 ± 0.01^a	0.21 ± 0.01^a	0.19 ± 0.02^{ab}	0.18 ± 0.01^{ab}	0.22 ± 0.02^b
16:1n-7	0.20 ± 0.01^a	0.20 ± 0.00^a	0.24 ± 0.02^a	0.22 ± 0.01^a	0.28 ± 0.02^b	0.26 ± 0.01^b	0.28 ± 0.02^b	0.44 ± 0.05^c
17:1n-8	0.05 ± 0.00^a	0.05 ± 0.02^a	0.06 ± 0.01^a	0.05 ± 0.01^a	0.07 ± 0.01^a	0.07 ± 0.01^a	0.07 ± 0.01^a	0.12 ± 0.01^b
18:1n-9	1.25 ± 0.10^a	0.80 ± 0.05^b	1.33 ± 0.12^a	1.22 ± 0.10^a	1.58 ± 0.08^c	1.80 ± 0.03^d	1.47 ± 0.08^c	2.70 ± 0.16^c
18:1n-7	1.03 ± 0.01^a	0.77 ± 0.01^b	0.65 ± 0.19^b	0.73 ± 0.08^b	0.86 ± 0.10^b	0.95 ± 0.06^{ab}	0.46 ± 0.02^c	1.24 ± 0.11^d
20:1n-9	0.01 ± 0.00^a	0.05 ± 0.07^b	0.02 ± 0.00^a	0.00 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.00 ± 0.00^a
20:1n-7	2.98 ± 0.27^a	1.24 ± 0.13^b	2.59 ± 0.20^a	2.85 ± 0.16^a	2.98 ± 0.07^a	3.38 ± 0.01^c	1.91 ± 0.05^d	4.17 ± 0.20^e
22:1n-11	0.05 ± 0.03^a	0.04 ± 0.00^a	0.04 ± 0.03^a	0.05 ± 0.02^a	0.09 ± 0.04^{ab}	0.08 ± 0.01^b	0.02 ± 0.00^a	0.04 ± 0.02^a
22:1n-9	0.38 ± 0.04^a	0.57 ± 0.05^b	0.45 ± 0.06^c	0.48 ± 0.02^c	0.47 ± 0.03^c	0.50 ± 0.02^{bc}	0.39 ± 0.05^a	0.42 ± 0.11^{bc}
Σ Monounsaturated	5.93 ± 0.25^a	3.71 ± 0.17^b	5.35 ± 0.75^c	5.61 ± 0.37^{ac}	6.32 ± 0.26^d	7.03 ± 0.06^e	4.60 ± 0.23^f	9.13 ± 0.49^g
16:4n-3	0.44 ± 0.03^{ac}	1.83 ± 0.07^b	0.48 ± 0.15^{ac}	0.39 ± 0.02^a	0.39 ± 0.01^a	0.48 ± 0.04^c	0.66 ± 0.02^d	0.43 ± 0.02^{ac}
18:2n-6	0.06 ± 0.01^a	0.30 ± 0.06^b	0.10 ± 0.04^a	0.08 ± 0.04^a	0.08 ± 0.02^a	0.16 ± 0.02^c	0.13 ± 0.01^c	0.22 ± 0.02^d
18:3n-6	0.05 ± 0.01^a	0.07 ± 0.00^b	0.05 ± 0.01^a	0.05 ± 0.01^a	0.06 ± 0.01^{ab}	0.06 ± 0.00^{ab}	0.03 ± 0.00^c	0.08 ± 0.01^b
18:3n-3	0.07 ± 0.05^{ab}	0.05 ± 0.00^a	0.04 ± 0.01^a	0.04 ± 0.00^a	0.08 ± 0.03^{ab}	0.08 ± 0.01^b	0.04 ± 0.00^a	0.08 ± 0.01^b
18:4n-3	0.16 ± 0.11^a	0.22 ± 0.00^b	0.08 ± 0.02^a	0.10 ± 0.01^a	0.20 ± 0.06^{ab}	0.23 ± 0.04^b	0.08 ± 0.03^a	0.14 ± 0.05^c
20:2n-6	0.07 ± 0.00^a	0.18 ± 0.05^b	0.11 ± 0.04^a	0.08 ± 0.00^a	0.08 ± 0.00^a	0.20 ± 0.01^b	0.17 ± 0.01^b	0.27 ± 0.01^c
20:4n-6	0.98 ± 0.01^a	1.17 ± 0.10^b	0.58 ± 0.16^a	0.65 ± 0.03^c	0.89 ± 0.12^b	1.19 ± 0.02^b	0.46 ± 0.07^c	1.08 ± 0.05^a
20:3n-3	0.07 ± 0.00^a	0.17 ± 0.02^b	0.12 ± 0.07^{ab}	0.08 ± 0.02^a	0.13 ± 0.04^b	0.18 ± 0.02^b	0.23 ± 0.03^c	0.33 ± 0.06^d
20:4n-3	0.04 ± 0.01^a	0.03 ± 0.02^a	0.05 ± 0.02^{ab}	0.05 ± 0.01^a	0.04 ± 0.01^a	0.07 ± 0.00^b	0.03 ± 0.00^a	0.14 ± 0.00^c
20:5n-3	5.12 ± 0.22^a	5.84 ± 0.08^b	5.68 ± 0.51^{ab}	5.35 ± 0.57^{ab}	5.53 ± 0.68^{ab}	5.55 ± 0.27^{ab}	6.16 ± 0.25^c	7.26 ± 0.28^d
22:4n-6	0.02 ± 0.00^a	0.12 ± 0.04^{bc}	0.02 ± 0.02^a	0.01 ± 0.00^a	0.08 ± 0.02^b	0.03 ± 0.01^a	0.05 ± 0.02^a	0.16 ± 0.09^c
22:5n-6	0.19 ± 0.05^{ab}	0.17 ± 0.01^a	0.10 ± 0.04^b	0.11 ± 0.04^b	0.19 ± 0.02^a	0.22 ± 0.11^c	0.11 ± 0.01^b	0.31 ± 0.04^d
22:5n-3	0.22 ± 0.02^a	0.44 ± 0.01^b	0.25 ± 0.08^{ac}	0.29 ± 0.02^c	0.32 ± 0.01^c	0.26 ± 0.02^a	0.12 ± 0.05^d	0.39 ± 0.06^b
22:6n-3	16.09 ± 0.42^a	10.77 ± 0.22^b	15.57 ± 1.10^{ac}	14.88 ± 1.38^c	15.74 ± 1.73^{ac}	14.75 ± 0.35^c	16.68 ± 1.15^a	17.10 ± 0.98^a
Σ Polyunsaturated	23.58 ± 0.50^a	21.34 ± 0.60^b	23.23 ± 1.62^a	22.16 ± 2.01^{ab}	23.83 ± 2.49^a	23.47 ± 0.33^a	24.94 ± 1.53^a	28.00 ± 1.60^c
Σ Total FA	45.18 ± 0.74^{ac}	38.35 ± 1.14^b	44.54 ± 2.43^a	42.80 ± 3.54^a	45.56 ± 3.40^{ac}	47.52 ± 0.56^c	46.45 ± 2.63^{ac}	55.08 ± 2.68^d

Different superscript letters within rows represent significant differences ($P < 0.05$).